

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
VINCLOZOLIN

Chemical Code # 002129, Tolerance # 00380
SB 950 # 123

Original version: July 30, 1986
Revised 12/20/88, 11/27/89, 8/23/90, 3/29/91, 7/9/91, 9/01/92, 11/25/92, 8/17/93, 12/23/94,
11/27/95, 4/10/96, 7/24/97, and 7/20/98

I. DATA GAP STATUS

Chronic, rat:	No data gap, possible adverse effects
Chronic dog:	No data gap, no adverse effect
Oncogenicity, rat:	No data gap, possible adverse effects
Oncogenicity, mouse:	No data gap, possible adverse effects
Reproduction, rat:	No data gap, possible adverse effects
Teratology, rat:	No data gap, possible adverse effects
Teratology, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, possible adverse effect
Chromosome effects:	No data gap, no adverse effect
DNA damage:	No data gap, no adverse effect
Neurotoxicity:	Not required at this time

Note: Toxicology one-liners are attached

Note: In one liner volume/record number citations below:

** indicates acceptable study

Bold face indicates possible adverse effect

This revision considers all submissions with record numbers up to 160342 (Document 380-154). These include all records on file as of 7/01/98. Record numbers greater than 900,000 have also been reviewed, where appropriate. Aldous, 7/20/98.

Note: these pages contain summaries only. Individual worksheets may identify additional effects.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

CHRONIC AND COMBINED CHRONIC/ONCOGENICITY, RAT

NOTE: Collectively, the chronic studies under DPR Record Nos. 130565 and 130567, together with oncogenicity study No. 130571, indicate a NOEL of 25 ppm. Pivotal records for NOEL determination are Record Nos. 130571 and 130567. Record No. 130571 indicated an LEL of 50 ppm, based on findings in both sexes in a study with group sizes of 50/sex/group. Record No. 130567 found no chronic effects at 25 or 50 ppm. Due to the smaller group sizes of the latter study (20/sex/group), the 50 ppm level is considered to be an LEL rather than as a NOEL. The final data gap requirement was clarification of possible gender differences in clinical observations at onset of dosing. This is now addressed (immediately below). Aldous, 12/22/94, 9/29/95, and 4/2/96.

****380-137 and -138 144256 and 144258** Mellert, W. and B. van Ravenzwaay, "Response of BASF to CDPR's review of the two chronic/oncogenicity studies (BASF Project Nos. 71S0375/88105 and 71S0375/88026)". BASF Reg. Document No. 95/5185. Record No. 144256 refers to Record No. 130565 (BASF Project No. 71S0375/88026). Record No. 144258 refers to Record No. 130571 (BASF Project No. 71S0375/88105). Record No. 144258 also has individual clinical observation data for a 3-month dietary study not previously submitted: BASF Project No. 31S0375/88034. Date of cover letter to reports: 1/18/96. Weekly "group observation data" show that different technicians performed clinical observations data for males and females in both of the long-term studies. One of these studies reported only males affected and the other study reported only females affected at or above 3000 ppm with signs of "reduced general state" in the first 1-2 weeks of treatment. A third study (3-month rat dietary study performed in the same facility) had the same technician examining both sexes, who reported "reduced general state" during the first week of study in both sexes at 3000 ppm. It may be concluded that this clinical observation represented a subtle and temporary effect, which was manifest in both sexes at or above 3000 ppm. The data gap for rodent chronic studies is now filled (see primary reviews for descriptions of "possible adverse effects"). Aldous, 4/10/96.

380-131 136410 van Ravenzwaay, B., "Summary : Toxicological assessment of the technical active ingredient VINCLOZOLIN". Report was dated Nov. 1994. This report (evidently not containing new data) may be of value to risk assessors preparing toxicology profiles. This "Summary" followed a cover letter containing a rebuttal against DPR's classification of the "rat chronic" data gap as "not filled". Some information from this "Summary" was included in the DPR response, made on this date (Aldous, 9/29/95).

380-116 130565 Mellert, W., "Chronic toxicity study with Reg. No. 83 258 (Vinclozolin) in rats. Administration in the diet for 24 months", BASF Aktiengesellschaft, Department of Toxicology, Ludwigshafen, May 3, 1994. Lab Proj. ID 71S0375/88026. Vinclozolin, purity 99.3%, was administered in the diet at concentrations of 0, 150, 500, 1500 or 4500 ppm to 20 Wistar rats/sex/group for 24 months. No NOEL was established in this study (see the follow-up low-dose study under DPR Record No. 130567 for a relevant NOEL). The most definitive effects observed down to 150 ppm included lens degeneration (M), testicular interstitial cell tumors and calcification of the tubuli, prostate gland interstitial fibrosis, and lipidosis of ovarian interstitial cells. See paragraph at top of this section regarding fulfillment of rat chronic data gap considering this and related studies, based upon a reconciliation of apparent disagreement between this study and Record No. 130571 (BASF Lab. Proj. ID 71S0375/88105) as to which sex is preferentially affected by clinical signs of poor "general state" in the early phase of

treatment. The "**possible adverse effects**" are the same as elaborated in the review of the oncogenicity study (Record No. 130571) [tumor formation in testes, liver, ovaries, and adrenal cortex; also, increased formation of cataracts]. The "possible adverse effect" of highest concern is the effect that vinclozolin may have on human reproductive systems. The high dose exceeded MTD criteria, based on changes in food and water consumption, body weights, and clinical signs. Kishiyama and Aldous, 12/22/94. (Data gap was subsequently filled; see upgrade above)

380-077 091643 (An early interim report of Record No. 130565, above). Worksheet by Aldous, 1/9/91.

380-082 092436 Essentially an exact duplicate of 091643, above.

380-091 115985 Draft pathology report of Record No. 130565, above). Worksheet by Aldous, 8/27/92.

380-091 115988 Additional preliminary data to Record No. 130565, above. Brief worksheet by Aldous, 8/27/92.

380-117 130567 Mellert, W., "Chronic toxicity study with Reg. No. 83 258 (Vinclozolin) in rats; administration in the diet for 24 months", BASF (Ludwigshafen), Lab Proj. ID 71S0375/88109, May 3, 1994. This was a supplemental low-dose range study corresponding to a higher dose range study in Record No. 130565. Vinclozolin, purity 99.3%, was administered in the diet at concentrations of 0, 25, or 50 ppm to 20 Wistar rats/sex/group for 24 months. NOEL = 50 ppm (approximately 2.4 mg/kg/day for males and 3.2 mg/kg/day for females). **Acceptable supplemental study**, with no treatment effects. (Kishiyama and Aldous, Dec. 1, 1994).

380-107 125385 (interim report for Record No. 130567, above): no review.

380-091 115991 (no author). [Exploration of mechanism associated with study 380-077:091643]. "Preliminary information of selected findings of the Reversibility Study II of Reg. No. 83 258 (Vinclozolin) in Wistar rats; Dietary administration for 3 months and 8-week recovery period, Project No.:99S0375/88114". BASF Aktiengesellschaft, Department of Toxicology, Ludwigshafen. (Interim report; cover letter dated 6/15/92). Male and female rats were dosed with 0 or 4500 ppm vinclozolin for 3 mo, then taken off treatment for 8 wk. Plasma hormone levels were assayed immediately following treatment, and again after the 8 wk recovery period. Male rats had remarkable increases in 3 anterior pituitary hormones as a result of treatment (ACTH, LH, and FSH). Testosterone, DHEA, corticosterone, and aldosterone were also elevated due to treatment. All these parameters were normal following the recovery period. In females there was also an increase in ACTH, but a reduction in adrenal cortical hormones. Females also had elevated LH, but no change in assayed LH-regulated hormones. These are useful mechanistic data, consistent with alteration by vinclozolin of a hormonal feedback loop. **Not acceptable (mechanistic study, not peer reviewed, techniques not detailed enough), but useful information.** Aldous, 9/01/92.

056 071711 "First preliminary information of ophthalmological finding with REG. NO. 83 258 (Vinclozolin) in rats after oral administration". BASF AG, Department of Toxicology. Preliminary report dated 10/13/88. Two groups of Wistar rats were tested: the first study involved 10/sex/treatment at doses of 0, 300, 1000, and 3000 ppm in diet over an 82-day period. The

second group was part of a chronic study, [full report of that study is Record No. 130565, above]. Only a single interim ophthalmological examination had been done to date for the chronic study (day 94). Both studies found cataracts in females (3000 or 4500 ppm by 82 or 94 days, respectively). One male developed cataracts by day 94 at 4500 ppm. Both studies showed an increase in lens lesions, which would be expected to progress to cataracts over time. An apparent "92-day NOEL" of 150 ppm was observed for lens lesions in the portion of the chronic study completed so far. These ophthalmological lesions are a "**possible adverse effect**". C. Aldous, 12/5/88 (updated by Aldous, 11/16/94).

380-060:072996 (protocol to Record No. 130565, above).

380-091 115992 Neumann, F., "Early indicators for carcinogenesis in sex-hormone-sensitive organs". Mutagenicity. Res. 248:341-356 (1991). The relevant part of this brief review discusses a negative feedback system involving the hypothalamus, anterior pituitary, and testicular interstitial cells. Compounds with antiandrogenic effect appear to compete with the normal negative feedback effect of testosterone at hypothalamic receptors, resulting in excessive release of gonadotropin-releasing hormone from the hypothalamus. This is followed by excessive release of hormones from the anterior pituitary (notably of LH), resulting in excessive stimulation of testicular interstitial cells. The overstimulation results in interstitial cell tumors. Useful ancillary information: no DPR worksheet. Aldous, 8/31/92.

380-091 115993 Roberts, S.A. et al., "SDZ 200-110 induces Leydig cell tumors by increasing gonadotropins in rats". SDZ 200-110 caused an increase in interstitial cell tumors in Sprague-Dawley rats. There was also a dose-related increase in serum LH and FSH levels, without a sustained alteration in testosterone levels. Useful ancillary information: no DPR worksheet. Aldous, 8/31/92.

380-091 115994 Pavone-Macaluso, M., et al., "Is there a role for pure antiandrogens in the treatment of advanced prostatic cancer?", Uro-Oncology: Current Status and Future Trends, pp. 149-157, = 1990 Wiley-Liss, Inc. A "pure antiandrogen", Flutamide, blocks hypothalamic androgen receptors (resulting in inhibition of the normal negative feedback of the hypothalamus on pituitary release of LH). Flutamide is used in treatment of prostate cancer in man. In intact rats, Flutamide markedly increases testosterone levels, but there is no sustained increase on chronic exposure in man. The inference to BASF scientists (see p. 2 of cover letter at beginning of this volume) is that flutamide is a functional analog of vinclozolin, either one of which would have a far lesser effect on interstitial cell changes in man than in rats. Aldous (no DPR worksheet), 9/1/92.

SUBCHRONIC, RAT

****380-138 144258** (Part 2) Mellert, W., "Study on the oral toxicity of Reg. No. 83 258 (vinclozolin) in Wistar rats; Administration in the diet over 3 months", BASF Aktiengesellschaft, Ludwigshafen, March 5, 1993. Project ID 31S0375/88034. BASF Registration Document No. 93/10191. Ten rats/group received 0, 300, 1000, or 3000 ppm vinclozolin (99.2%) in diet for 3 months. Parameters studied included ophthalmology at 42-52 days and at termination, in addition to normal parameters for subchronic studies. No NOEL exists in the dosage range: 300 ppm females had enlarged, discolored adrenals (without associated histopathology at that dose level) and lens striations. Major histopathology findings at 1000 to 3000 ppm included adrenal zona fasciculata hypertrophy and vacuolation, testicular Leydig cell hyperplasia, pancreatic

acinar vacuolation, hepatocellular cloudy swelling and single cell necrosis. Findings usually limited to 3000 ppm included increased water consumption; general decrease in white blood cell counts, and in RBC counts, Hb, and HCT; increased circulating levels of globulins, triglycerides, and cholesterol in 3000 ppm females; general decrease in circulating salt concentrations (N/A+, K+, and Ca++); and cataracts in two 3000 ppm females. Findings such as lens striations and adrenal enlargement/discoloration at 300 ppm, as well as cataractogenesis at 3000 ppm are "possible adverse effects". This study tends to confirm effects already noted at comparable or lower effect levels in longer term studies. Study is **acceptable**. Aldous, 4/10/96.

380-056 071709 "Comparative study on the cataractogenic activity of procymidone, vinclozolin and dichlozoline in rats". Sumitomo Chemical Co., Ltd. (dated after 2/6/77). (Subchronic study for eye effects only.) Male Sprague-Dawley rats were fed diets of 0, 750, 1500, 3000, or 5000 ppm vinclozolin (95%) for 6 months, 20 rats/group except for 5000 ppm group, which had 10 rats/group. Rats were examined on alternate weeks for ophthalmological changes. No other analyses were made. After 26 weeks, 1500, 3000, and 5000 ppm vinclozolin groups had 2/18, 9/18, and 6/9 rats with cataracts, respectively, vs 0/20 in controls and 0/19 in the 750 ppm group. Apparent NOEL = 750 ppm, based on above studies. Data suggest a **"possible adverse effect"**. Study is **supplemental by design**, and **not upgradeable** to fill SB-950 data requirements (study was limited in length and scope, hence not a "chronic" study; test article does not appear to be technical vinclozolin; no indication of control methods to avoid potentially confounding circumstances (intensity of lighting and location of test groups with respect to light sources); no examination of other aspects of animal treatment response. C. Aldous, 12/19/88.

380-139 147391 Mellert, W., K. Deckardt, C. Gembardt, W. Kaufmann, and B. Hildebrand, "Reg. No. 83 258 (Vinclozolin) - Reversibility of selective findings in Wistar rats: Dietary Administration for 3 months and 1-month and 3-month recovery periods", BASF AG, Ludwigshafen, 12/18/95. Laboratory Project ID: 39S0375/88116. Ten rats/sex/dosage group were allocated to each of three treatment/recovery regimens: (1) 3 months treatment without recovery, (2) 3 months treatment with 1 month recovery, or (3) 3 months treatment with 3 months recovery. Dose levels were 0, 1000, or 4500 ppm. Study parameters included hematology, clinical chemistry, ophthalmology, and gross necropsies. Eight/sex/dose/sacrifice interval were then examined by standard histopathologic techniques for selected organs known to be responsive to vinclozolin toxicity. Up to two/sex/dose/sacrifice interval were evaluated by electron microscopy for selected target tissues. The study was designed to evaluate the rate and extent of recovery of vinclozolin pathology. High dose rats had initial clinical signs of "poor general state", but had a normal appearance thereafter. High dose males had marked food consumption decrements and associated body weight decrements of about 100 g, with nearly complete recovery by the end of the 3-month recovery period. Clinical chemistry was normal, except for a notable increase in γ -glutamyltransferase during the treatment period, to which no particular histopathology appeared to be associated. Adrenal weights were remarkably elevated at the end of the dosing period in both sexes. A slight elevation continued through the 3-month recovery period in males. Liver weights were remarkably elevated, particularly in females, with rapid return to normal weights after cessation of treatment. Testes weights were elevated and male accessory organ weights were markedly diminished by treatment. Several accessory gland weight decrements were statistically and biologically significantly reduced after 1 month recovery (epididymides, seminal vesicles, and prostate). Prostate weights appeared still meaningfully reduced after 3 months of recovery. Histology showed varying rates and degrees of recovery. Liver centrilobular hypertrophy was seen in nearly all treated rats at the end of the treatment period, however after 1 month of recovery, only one 1000 ppm male and two 4500 ppm males

presented hypertrophy (minimal grade). Adrenal cortex had a marked lipid vacuolation, which was largely reversed by 1 month of recovery. In contrast, vacuolar degeneration of the adrenal cortical zona reticularis was prominent after treatment, clearly present after 1 month of recovery, and present to slight degree after 3 months of recovery. Pancreatic acinar cell vacuolation was well established at the end of the treatment period, with recovery over time to the extent that at 3 months, only males were affected, with minimal severity of lesions. In the eyes, cataract (ophthalmologic diagnosis) or lenticular degeneration (histopathological designation) proved to be an irreversible event. Aldous, 7/16/97.

380-091 115990 Preliminary report for Record No. 147391, above.

CHRONIC, DOG

****380-053 070946** "Report on the study of the toxicity of Reg. No. 83 258 (Vinclozolin) in beagle dogs after 12-month administration via the diet". BASF Dept. of Toxicology Project No. 3300165/8444, 12/17/87. Vinclozolin, technical., 98.9% purity, in diets of purebred beagles (6/sex/group) at 0, 35, 75, 150, and 1500 ppm; achieving average dosages of approximately 0, 1.2, 2.5, 5, and 50 mg/kg/day (estimated from food intake). NOEL = 2.5 mg/kg/day (based on increased adrenal cortical weight in females; in males, relative testicular weight increased, and there was a slight increase in prostate atrophy). These signs were more pronounced at the 50 mg/kg/day level, where there was also diffuse hyperplasia of testicular interstitial cells, diffuse atrophy of prostate, and lipid change in adrenals. Signs of slight anemia at 50 mg/kg/day (slight increase in reticulocyte counts, slight increase in hemosiderin deposition in liver, other minor changes in hematologic parameters). Original review found study not acceptable (more complete description of adrenal cortical lesions was needed). A subsequent rebuttal response clarified the lesions as lipid storage in the Zona fasciculata (see below). Report is now **acceptable. No adverse effect is indicated.** C. Aldous, 12/19/88, 11/20/89.

380-060 (no record number). Addendum to dog chronic study 053:070946. "Report on the study of the toxicity of Reg. No. 83 258 (Vinclozolin) in beagle dogs after 12-month administration via the diet". BASF Dept. of Toxicology, 12/17/87. The "lipid increase" in the adrenal cortex which was described in the original report was clarified in this rebuttal statement as increased storage of lipoids in cells of the Zona fasciculata, which was the only area of the cortex which underwent identifiable changes. With this clarification, the study is upgraded to **acceptable** status. Aldous, 11/20/89.

380-064 074822 "BASF response to EPA's April 1989 review of the one year dog feeding study with Vinclozolin (Ronilan)". These supplementary data addressed concerns of the EPA reviewer regarding the study designated by CDFA as 053:070946. Although the concerns of the EPA reviewer were not listed as requirements for CDFA acceptability, the submission of these data and clarifications helps to make the report more complete. This record includes original signed QAU and GLP statements; historical data on corneal opacity incidence before treatment (all treatment groups, 12 studies) or after treatment (controls only, in 9 of the above 12 studies which were complete by the time of the response); historical control reticulocyte data; clarifications of hematology methods and results; also on pathology methods and results, with particular attention to male reproductive structures (in which no treatment-related findings were noted). No change in CDFA status (acceptable). C. Aldous, 11/27/89. (No worksheet for this record).

380-005 981444 Hofmann, H. T. and R. Munk "Report on the toxicological testing of 3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidin-2,4-dione in a three month feeding trial on the dog", 4/28/75. BASF Report C14. Four beagles/group were dosed with 0, 100, 300, 1000, or 2000 ppm vinclozolin technical (purity not specified) in diet for 3 months. Estimated vinclozolin intakes were 0, 3, 9, 30, and 60 mg/kg/day (using default intake conversion factors of Zielhuis and van der Kreek, 1979). No clinical signs were noted. One high dose male rejected a substantial portion of its diet, however no body weight differences were found in any groups. Ophthalmology was negative. High dose females had reductions of Hb concentrations and RBC counts: this was considered by investigators as having "no pathological significance". The apparent NOEL of 9 mg/kg/day was based on cholestasis, affecting two males and two females at 1,000 ppm. At 2,000 ppm, all animals displayed a moderate cholestasis. No other histopathology was considered to be treatment-related by the pathologist. This brief report is unacceptable (test article is not characterized, report contains no tables for review, no GLP verification). No DPR worksheet was made. Aldous, 6/19/97.

ONCOGENICITY, RAT

NOTE: Record 130571 should be considered the single definitive rat oncogenicity study, since (1) its results are consistent with the results of the major recent rat chronic study (Record No. 130565) with respect to the major long-term findings, and (2) the 1977 study below (Record No. 035308) failed to detect several of these effects. Aldous, Dec. 1, 1994.

****380-118 130571** Mellert, W., "Carcinogenicity study with Reg. No. 83 258 - Vinclozolin in Wistar rats. Administration in the diet for 24 months", BASF Aktiengesellschaft, Department of Toxicology, Ludwigshafen, May 2, 1994. BASF Lab. Proj. ID 71S0375/88105. Vinclozolin, purity 99.3%, was administered in the diet at 0, 50, 500 and 3000 ppm to 50 Wistar rats/sex/group for two years. In addition to oncogenicity assessment, study included special attention to male sexual accessory tissues, and periodic ophthalmological examinations. Body weight and food consumption were markedly reduced for high dose groups. Also, high dose males appeared to be in poor general condition for the first two weeks of treatment, with normal appearance thereafter. Together, these changes suggest that the MTD was exceeded at 3000 ppm. No NOEL was found [findings at 50 ppm included eosinophilic foci in liver (M), degeneration of the lens (F), and lipidosis of ovarian interstitial cells (F)]. Several tumors arose in reproductive tissues, including testicular interstitial cell tumors (chiefly adenomas), prostate gland adenomas, ovarian stromal cell tumors (generally benign), and uterine adenocarcinomas. Adrenal cortical adenomas were sharply elevated in high dose females. Marginal increases of hepatocellular tumors in 500 to 3000 ppm males and in 3000 ppm females were considered by this reviewer to indicate treatment effects. Other common findings at 3000 ppm and often 500 ppm included hepatocellular hypertrophy, lipidosis of adrenal cortex, aggregates of foam cells in lungs, serous fluid accumulation in anterior chamber of the eye, and vacuolation of pancreatic acinar cells. Also, there was widespread evidence of reduced responsiveness of testosterone-regulated tissues to testosterone. Changes included atrophy, reduced functionality, and/or oligospermia (or azoospermia) of testes, epididymides, seminal vesicles, coagulation glands, and prostate gland. Study indicates a **"possible adverse effect"**, based primarily upon tumors and lens effects. **Acceptable** oncogenicity study. Kishiyama and Aldous, 10/18/94.

380-024 and -025 035308, 035309 Leuschner, F., A. Leuschner, F. Hübscher, and W. Dontenwill, "Chronic oral toxicity of an oxazolidine derivative, Batch 83 258 - called for short "Oxa" - in the Sprague-Dawley rat". Laboratorium für Pharmakologie und Toxikologie [LPT],

12/15/77. Vinclozolin, 93% purity, in diets of Sprague-Dawley rats, 50/sex/group, at 0, 162, 486, 1458 or 4374 ppm for 130 weeks. **No adverse effects indicated.** A mild degree of liver necrosis appeared to be treatment-related in high dose females, but lower dosages were apparently not systematically examined to determine a NOEL for that effect. The apparent NOEL (excluding liver) is 486 ppm, based on dose-related body weight decrements in males and females. Additional information was provided in rebuttal/additional data in Vols. 41 and 44 to address some concerns raised in 10/7/85 CDFA review. Subsequently, record 380-081:096398 (see below) showed stability of vinclozolin in diet for at least 32 days at room temperature, following comparable dose preparation methods. C. Aldous, 10/7/85, 12/19/88, July 2, 1991. Study was considered upgradeable until Record No. 121890 made it clear that it will not be possible to verify dose levels achieved in this study. The study is now considered **not upgradeable**. Aldous, 8/17/93.

041 060668 Rebuttal comments for rat study 024:035308; Characterization of vinclozolin, including purity and maximum levels of major contaminants; specific brief responses to CDFA concerns listed in the 10/07/85 CDFA review; table of histopathology findings in liver; tissue inventory table. These data were considered in 12/19/88 review of study 024:035308 by C. Aldous.

044 062625 Additional data for rat study 024:035308. Late in-life individual observations and mode of death data for rats, and weekly summary data of rats dying on study. These data were considered in 12/19/88 review of study 024:035308 by C. Aldous.

005 981456 Partial duplicate of 024:035308.

060 072954 (Appendix 5, associated with BASF's 1/19/89 rebuttal to CDFA concerns about rat and mouse oncogenicity studies completed in 1977). This is a 1-page record containing a single table, demonstrating satisfactory storage stability of vinclozolin [technical] over periods up to 2 years at temperatures from 30° to 50°C. The preceding Appendix (Number 4) was not given a CDFA Record Number, but is relevant. Appendix 4 was cited in the 1/19/89 Rebuttal as evidence that test article incorporated into meal was stable for at least 32 days at room temperature (see p. 5 of rebuttal at front of Document No. 380-060). Appendix 4 contains only one page, which is a table entitled "Concentration Control/Homogeneity in Carrier". The table does not appear to address storage stability. Thus there is no acceptability status change for either the rat or mouse oncogenicity studies of 1977. C. Aldous, 11/27/89.

380-081 096398 [addendum to document #'s 380-024, -025, and -026; record #'s 035308 to 035310 (rat and mouse oncogenicity studies, respectively). New data include a table prepared at the BASF analytical laboratory, showing stability of 50 mg/kg nominal concentration of vinclozolin in diet for at least 32 days at room temperature. Also, there is a statement indicating that essential features of treated diet preparation were similar between LPT and BASF. Only one item remains outstanding for these two studies to be upgraded to acceptable status: dose preparation records from the original studies. Photocopies from the original laboratory notebooks are acceptable. Aldous, July 2, 1991. (See, however, Record No. 121890, below).

380-088 111354 and 111355 Addendum to documents 380-024 and -025 (rat), and 380-026 (mouse), and to records 035308-035309 (rat), and 035310 (mouse). Cover letter for this submission was dated 11/22/91. Diet preparation records for these studies are no longer

available. Ongoing studies are in progress for rat chronic and oncogenicity, and for mouse oncogenicity study types. Aldous, 1/08/92.

380-102 121890 Tobia, A.J. [information relating to Document No. 380-024, Record No. 035308 (**rat**) and Document No. 380-026, Record No. 035310 (**mouse**)]. Title [of present submission]: "Review of the evidence in support of the upgradeability of the oncogenicity rat and oncogenicity mouse studies for vinclozolin". BASF Corp., date [of present submission], Feb. 11, 1993. Submissions attest to the stability of test article, and of treated diets under use conditions. Acceptable homogeneity was demonstrated under preparation conditions of BASF facilities. General directions for treated diet preparation at the laboratory which performed the above rat and mouse studies were shown to be similar to general directions used more recently at BASF. There were no specific instructions as to how much a.i. was added to a given amount of diet in the cited Laboratory of Pharmacology and Toxicology (LPT) studies. It was noted that individual records for weights of test compound employed in diet preparation are no longer available. Figures show that rats and mice of the LPT studies cited above had body weight decrements comparable to those of corresponding groups in the current rat and mouse studies recently performed at BASF. Data do not supply sufficient documentation of actual dose levels administered to suffice in lieu of dose preparation made during the progress of the studies. Definitive data cannot be obtained to fill the data gap retrospectively. Study status for the above cited studies should be changed to "**not upgradeable**". Aldous, 5/03/93.

380-140 147394 van Ravenzwaay, B., "Assessment and mode of enhancement of adrenal cortical tumors in female Wistar rats treated with vinclozolin", 12/27/95 (Sponsor Report #95/11206). Adrenal cortical adenomas had been previously observed as high dose responses in female rats [see especially DPR Record No. 130571 (BASF Lab. Proj. ID 71S0375/88105)]. This assessment of adrenal cortical toxicity notes that tumors arose at excessive dose levels, whereas lower dose levels also caused some cortical changes (enlargement and discoloration, with reversible lipidosis and vacuolation). Findings at high doses included persistent lipogenic pigment, reflecting focal dystrophia. These findings, seen at 3000 to 4500 ppm, but not at 1000 ppm or below, were considered by the author to represent a threshold for irreversible adrenal cortical toxicity. Previous studies were cited which found reductions in corticosterone levels at high vinclozolin dose levels, accompanied by markedly increased ACTH concentrations. Further studies may be relevant to assess the nature of the lipid material in cortical cells and the reason for its accumulation. Aldous, 6/26/97.

ONCOGENICITY, MOUSE

****380-119 130572** W. Mellert, "Carcinogenicity study with Reg. No. 83 258 (Vinclozolin) in C57BL mice. Administration in the diet for 18 months." BASF Aktiengesellschaft, Department of Toxicology, Ludwigshafen, May 4, 1994, Lab. Proj. ID 80S0375/88112. Vinclozolin, purity 99.3%, was administered in the diet at concentrations of 0, 15, 150, 3000 and 8000 ppm to 50 and 10 C57BL/6/JICO mice/sex/group for 18 months (main group) and 12 months (satellite groups), respectively. The main study control groups contained 100 mice/sex. Mean vinclozolin intake for the oncogenicity study was 0, 2.1, 21, 432, and 1225 mg/kg/day for males and 0, 2.8, 28, 557, and 1411 mg/kg/day for females. NOEL = 150 ppm [most definitive changes were in liver (focal necrosis, bile duct proliferation, and pigmentation of Kupffer cells), testes (hyperplasia of interstitial cells), male accessory tissues (atrophy of seminal vesicles and coagulation glands), adrenal cortex (lipidosis), and uterus (atrophy)]. **Acceptable.** The increase in hepatocellular

tumors, principally carcinomas, most abundant in 8000 ppm females, is a **possible adverse effect**. There were modest increases in hemangiosarcomas in livers of 3000 to 8000 ppm males and in 8000 ppm females, evidently secondary to other liver pathology. Four of the 5 liver hemangiosarcomas in 3000 to 8000 ppm mice were associated with hepatocellular carcinomas, 3 of them being found in the same lesion. The carcinomas are the characteristic neoplastic responses of mice to vinclozolin, and hemangiosarcomas do not appear to arise independently. The 8000 ppm dose clearly exceeded "MTD" criteria, indicated by decrements in lifespan, food consumption, and body weights. Premature deaths at 3000 ppm and especially at 8000 ppm were related to erosions and ulcerations of the glandular stomach: often these deaths occurred early in the study. Kishiyama and Aldous, 11/29/94; re-examination by Aldous, 5/20/97.

380-096 119819 Interim report for Record No. 130572, above. Provisional examination by Aldous on 5/17/93, no worksheet.

380-108 125389 Interim report for Record No. 130572 (see above), no review.

380-026 035310 "Oral toxicity of an oxazolidine derivative, Batch 83 258 - called for short "Oxa" - in NMRI mice". Laboratorium für Pharmakologie und Toxikologie, 12/15/77, rev. 8/25/82. Vinclozolin, 93% purity, in diets of NMRI mice, 50/sex/group, at 0, 162, 486, 1458 or 4374 ppm for 112 weeks. Apparent NOEL is 1458 ppm, based on lower body weights in 4374 ppm M and F (greater than 10% difference during much of the lifetime for 4374 ppm F), also significantly increased liver weights in 4374 ppm M and F, spleen siderosis and hyperplasia in 4374 ppm M. Several concerns noted in the 10/11/85 CDFA review were addressed in rebuttal/additional data in Vols. 41 and 42. Subsequently, record 380-081:096398 (see above, under rat oncogenicity) showed stability of vinclozolin in diet for at least 32 days at room temperature, following comparable dose preparation methods. **No adverse effects are indicated:** a slightly increased incidence of lung adenomas or carcinomas in high dose females was considered prior to the 3/29/91 CDFA review to be a "possible adverse effect". Additional data showed that there was insufficient evidence of a treatment effect to ascribe changes to vinclozolin (see 079:095608, below). Also, some apparent increases in leukemias in both sexes were initially considered by CDFA to be possible treatment effects. This concern was resolved considering records [380-060] 072955, 072956 (see below) and other records. C. Aldous, 10/11/85, 12/19/88, 11/22/89, 3/29/91, and July 2, 1991. Study was considered upgradeable until Record No. 121890 made it clear that it will not be possible to verify dose levels achieved in this study. The study is now considered **not upgradeable**. Aldous, 8/17/93.

Note: see also one-liner for supplementary submission 380-088 111354 and 111355, above under "Oncogenicity, rat".

380-060 072955, 072956 (Supplementary to 380-026:035310). Record #072955 is Appendix 10A: "Discussion of historical control data for NMRI mice with special attention to lymphomas and leukemias". This discussion presented justifiable reasons for combining lymphomas and leukemias for analysis, in which case only males reflected an increase in these tumors. When historical control data for these common tumors were considered, it appeared appropriate to not consider these tumor incidences to reflect "possible adverse effects". Record #072956, Appendix 10B, is entitled "Summary Toxicological Assessment of the Technical Active Ingredient Vinclozolin". Pages 41-43 deal with mouse oncogenicity issues, however there were no new data in this submission. The mouse study still has one "possible adverse effect" (lung

tumors), and the study is still **not acceptable** (needs dose preparation records plus surrogate analyses demonstrating stability of test article in feed). C. Aldous, 11/22/89.

380-079 095608 [addendum to 026:035310, "Oral toxicity of an oxazolidine derivative, Batch 83 258 - called for short "Oxa" - in NMRI mice". BASF Corporation, Laboratorium für Pharmakologie und Toxikologie, report dated 12/15/77, revised 8/25/82. The new submission is a compilation of EPA memos on vinclozolin, including a 2/14/85 memo from EPA pathologist, L. Kasza, who cited the recently completed independent re-evaluation of lung slides by Experimental Pathology Laboratory, Inc. (EPL). The submission also included the subsequent EPA Peer Review Panel conclusions, dated 6/17/85. The EPL pathologist found lower incidence of bronchiolar-alveolar tumors in the higher dose groups of females than were originally reported, and concluded that the data did not warrant the presumption that the test article was oncogenic under conditions of the study. The EPA Peer Review Panel agreed. Aldous, 3/29/91.

041 060669 Rebuttal comments for mouse study 026:035310; Federal Register determination on vinclozolin (Federal Register 51:3635-3637, 1/29/86), which summarized EPA justification for not considering vinclozolin as a mouse oncogen; FAO Plant Production and Protection Paper No. 77 (Rome, 1986), which indicated vinclozolin to be "negative for oncogenic potential in rats and mice"; characterization of vinclozolin, including purity and maximum levels of major contaminants; specific brief responses to CDFA concerns listed in the 10/11/85 CDFA review. These data were considered in 12/19/88 review of study 026:035310 by C. Aldous.

042 062907 Additional data for mouse study 026:035310. Late in-life individual observations and mode of death data for selected mice, and weekly summary data of mice dying on study. These data were considered in 12/19/88 review of study 026:035310 by C. Aldous.

005 981457 Partial duplicate of 026:035310.

005 981458 Apparently a duplicate record number for 981457, above.

016 981459 Exact duplicate of 026:035310. Retain both record numbers.

030 050859 (Ancillary information to 026:035310). This is a rebuttal from BASF dated Aug., 1983 to an EPA memorandum of 4/15/83, which had classified vinclozolin as oncogenic based on the mouse oncogenicity study. The primary focus of the rebuttal regarded lung tumor findings. BASF noted that lung tumors are widely considered to have no counterpart in human pathology, that the tumors were increased only slightly, and in one sex only. There was no apparent reason for a sex difference in lung tumor incidence. Data to demonstrate the variability of control lung tumor values were presented. Evidence was presented for considering the slightly increased incidence of hepatocellular tumors to be not treatment-related. The rebuttal gave only passing mention to leukemia incidence. C. Aldous, 10/13/88.

NOTE: Additional data relevant to mouse tumor data interpretation have since been reviewed (see 1-liner for 026:035310).

380-082 092434 Hildebrand, B., "Study on the oral toxicity of Reg. No. 83 258 (VINCLOZOLIN) in C57BL mice. Administration in the diet over 3 months". Project No. 53S0375/88054. BASF Aktiengesellschaft, 10/90. This is one of two subchronic studies in different mouse strains, to set dose levels for the eventual oncogenicity study. Dose levels were 0, 100, 1000, and 5000 ppm. High dose mice had decreased food consumption without markedly reduced b.w. High

dose levels were typically associated with elevated RBC parameters (elevated RBC counts, HCT, and Hb). High dose group liver weights were elevated, consistent with liver hypertrophy, fatty infiltration (peripheral), and focal necrosis (females only). Adrenals of 1000 and 5000 ppm groups, especially females, had adrenal lipoid vacuolation and pigmentation. Clinical chemistry, largely reflective of liver toxicity and/or altered nutritional status, included elevated ALT and ALP in high dose males, decreased serum glucose (high dose M), and marked, dose-related decreases in serum triglycerides and cholesterol in 1000 and 5000 ppm M and F. Aldous, no SB-950 worksheet, 7/3/91.

380-082 092435 Hildebrand, B., "Study on the oral toxicity of Reg. No. 83 258 (VINCLOZOLIN) in B6C3F1 mice. Administration in the diet over 3 months". Project No. 53S0375/88025. BASF Aktiengesellschaft, 10/90. This is one of two subchronic studies in different mouse strains, to set dose levels for the eventual oncogenicity study. Dose levels were 0, 100, 1000, 2500, and 5000 ppm. Findings were similar to those of the C57BL study, above (Record 92434), although in some respects less severe. Aldous, no SB-950 worksheet, 7/3/91.

REPRODUCTION, RAT

****380-095 119728** "Reproduction study with REG No 83 258 (Vinclozolin) in rats continuous dietary administration over 2 generations (2 litters in the first and 2 litters in the second generation)" by J. Hellwig, BASF AG, Department of Toxicology, Ludwigshafen. Report # 92/11251, dated 10/21/92. Test article was Reg. No. 83 258 (vinclozolin), 99.2% purity. Original protocol called for the test article to be administered in the diet through 2 generations with 2 litters per generation at 0, 50, 300, 1000, or 3000 ppm to 24 Wistar [Chbb = THOM (SPF)] rats per sex per group. Mean vinclozolin intake for F1a rats was 4.5, 27, 96, and 314 mg/kg/day for males and 5.0, 30, 105, 335 mg/kg/day for females (prior to the first gestation period for females). F2a and F2b pups were not produced at 1000 and 3000 ppm due to altered sex expression in parental animals. Owing to this, representative pups of F1b, F2a, and F2b groups were maintained on treated diet for 14 weeks (F1b pups, see p. 52) or 11 weeks (F2a and F2b pups) beyond weaning to evaluate persistence of observations seen in pups. These three groups were designated as FX, FY, and FZ animals, respectively. Investigators concluded (justifiably) that no definitive NOEL exists, based on slight decrements in epididymal weights in FY and FZ males at 50 ppm (without associated histopathology), and on the presence of one FY male with unilateral seminiferous tubule atrophy at 50 ppm. A control FZ male also had seminiferous tubule atrophy, suggesting that 50 ppm is a defensible NOAEL (not a strong case for functional changes at 50 ppm). Findings at doses as low as 300 ppm with apparent dose-response relationships included (1) Parental non-reproductive effects: lenticular degeneration (both sexes), slightly reduced RBC counts and reduced HCT in females, and (2) Reproductive effects: Leydig cell hyperplasia, testicular tubular cell atrophy, and reduced secretion in prostate and coagulation glands. Major findings at 1000 ppm and above included hypoplasia of the penis and hypospadias in virtually all males, and these males were infertile. At this dose, both sexes had lipidosis of the adrenal cortex. The F1 generation pups gained weight more slowly than controls or lower dose groups, and definitive sex determination of pups was not possible in neonates. At 3000 ppm, the host of responses included high mortality of neonates, marked reduction in pup growth, and development of female-like sexual characteristics including Mullerian ducts and a vagina-like orifice. The study is **acceptable**. Failure of F1 males to acquire normal anatomical and functional male characteristics, marked retardation in neonatal growth and survival at dose levels not commensurately toxic to adults,

and lenticular degeneration are principal **possible adverse effects**. (H. Green and C. Aldous, 8/17/93).

380-062 074314 Preliminary information regarding reproduction study 095:119728, above. Reviewed by D. Shimer and C. Aldous, 8/23/90.

380-060:072953 Protocol for reproduction study (See preliminary information in 380-062:074314, above).

380-077 091646 Preliminary information regarding reproduction study 095:119728, above. Reviewed by Aldous, 1/11/91.

380-083 092437 Preliminary information regarding reproduction study 095:119728, above. Aldous (no new review), July 3, 1991.

380-077 091645 Protocol changes regarding reproduction study 095:119728, above. The scope of the study was expanded over the original protocol due to unexpected findings in the study. (Aldous, 1/11/91, no CDFA "review" of protocol changes.)

380-092 116627 Draft report on reproduction study 095:119728, above (issued prior to QA evaluation). Aldous, 8/25/92.

380-120 130573 [follow-up study to the primary reproduction study: Record No. 119728], Hellwig, J., "Second reproduction toxicity study with Reg. No. 83 258 (Vinclozolin) in rats". Lab. Proj. ID 70R0375/88119. Continuous dietary administration over 2 generations", BASF Aktiengesellschaft, Ludwigshafen, May 2, 1994. The primary study had not established a NOEL at doses as low as 50 ppm, based on reduced epididymal weights without microscopic changes. Therefore this study utilized dose levels of 0, 20, and 40 ppm in diets of 25 Wistar rats/sex/dose in the F0 generation. The F0 rats were maintained through 2 littering periods, and the F1a offspring were designated as F1 parental rats (25/sex/group), producing F2a and F2b offspring. Selected pups from F1b, F2a and F2b littering periods (25/sex/group) were raised to adulthood. All these adults were examined grossly at termination, and weights of liver, adrenals, and (in males) testes and epididymides were taken. Other major observations recorded included food consumption, body weight gain, clinical observations, reproduction and delivery data, pup morphological and functional development observations, and gross examinations of pups not selected for rearing to adulthood. There was no consistent evidence of a treatment effect. This is an **acceptable ancillary study** to Record No. 119728, providing a NOEL of 40 ppm for reproductive effects. Kishiyama and Aldous, Dec. 1, 1994.

380-094 119723 van Ravenzwaay, B., "Discussion of prenatal and reproduction toxicity of Reg. No. 83 258 (Vinclozolin)", Nov. 1992. Several studies show that vinclozolin is anti-androgenic with a potency comparable to a pharmacologically important drug, flutamide. High doses of vinclozolin (4500 ppm) lead to about 3-fold increase in testosterone in males, and about 9-fold increases in luteinizing hormone (LH). Androgen-dependent tissues nevertheless do not mature in cases of exposure over the developmental lifetime. Exposures beginning after sexual maturity lead to reduced functionality of androgen-dependent tissues. Binding affinity experiments found vinclozolin to bind to androgen receptors nearly as strongly as the pharmacologically active anti-androgen, flutamide. Investigators concluded that vinclozolin competes with androgens at androgen receptors at several sites, including the hypothalamic cells which normally provide feedback inhibition of pituitary LH production. LH, in turn, stimulates testicular interstitial cell

production of testosterone. The markedly increased testosterone levels do not compensate for the inhibitory effects of vinclozolin on androgen-dependent tissues. As a result, pituitary cells producing LH (and to a lesser extent, those producing FSH) are overstimulated, as also are the testicular interstitial cells, resulting in hyperplasia of these tissues. Meanwhile, tissues normally responding to androgen stimulation do not respond to increased testosterone levels due to competition by vinclozolin, leading to arrested development and/or structural or functional deficits. Studies indicate that findings observed in rats would be expected in humans, with potency comparable to flutamide. Useful data, relevant to risk assessment, but not a FIFRA-style presentation. Aldous, 8/17/93.

380-027 035311 Leuschner, F. and F. Hübscher, "Chronic oral toxicity of an oxazolidine derivative Batch No. 83258 - called for short 'Oxa' - in a reproduction study covering three generations of Sprague-Dawley rats". Laboratorium für Pharmakologie und Toxikologie, 12/9/77. Vinclozolin (93% purity, batch 83258) tested in a 3 generation, 2 litter study at 0, 162, 486 and 1458 ppm in the diet; 20/sex/group. Apparent NOEL = 1458 ppm (no evidence of toxicity). Original review indicated "unacceptable" (no justification of dose levels, lacking full identification of test article, lacking analysis of dosing material). Additional information in volume 041 provided test article identification. Items needed for upgrade remaining: dose justification (including documentation to verify that nominal dosages were prepared in the original study), and retrospective dosed feed analysis. An additional item not specified in the 10/11/85 review is necessary for an upgrade of this study: an independent audit of body weight and organ weight data for 9-week F3 pups. C. Aldous, 10/11/85, 12/20/88.

041 060670 Rebuttal comments to 027:035311, above, plus composition of active ingredient, considered in 12/20/88 review.

043 062624 Clinical observations data for 027:035311, above, considered in 12/20/88 review. (These data were uniformly negative).

005 981445 Partial duplicate of 035311.

005 981446 (No such entry found in this volume. Probable typographical error in library data base. Apparently Rec. No. 981445, above, was intended.)

380-152 160338 Hellwig, J., C. Gembardt, and H. -P. Gelbke, "Reg. No. 83 258 - Pre-/postnatal toxicity study in Long Evans rats after oral administration (gavage)," BASF Aktiengesellschaft, Ludwigshafen, 1/21/98. BASF Reg. Doc. # 98/10078. Female Long Evans rats, 25/sex/group, were dosed by gavage with vinclozolin (99.4% purity) once daily from day 14 of gestation until day 3 *post partum*. Dose levels were 0, 1, 3, 6, 12, and 200 mg/kg/day. The primary objective was to evaluate feminization effects in male pups, such as altered anogenital distances (on days 2 and 22) and nipple development (on days 12 or 13 *postpartum*) resembling that of female pups. Pups were culled on day 2 by removing every other pup. Remaining male pups were maintained for about 180 days without breeding trials, then necropsied to evaluate reproductive structures and gross lesions. One F1 female/litter was also retained for the balance of the study, but these were not extensively studied. Treatment was well tolerated by dams. The high dose group suffered an increase of stillborn pups, plus an excess of deaths through the first 2 days *post partum*. Growth and survival did not vary between groups after that time. Anogenital distance was reduced in high dose males. There was a dose-related increase in appearance of male pups with areola/nipple development in the 12 and 200 mg/kg/day groups at days 12-13. These changes persisted in high dose males, but virtually disappeared in 12 mg/kg/day males at

termination. Several changes in reproductive structures were evident in-life, all limited to 200 mg/kg/day. Of these, hypospadias was most universal and persistent. Paraphimosis and hypoplasia of penis were observed in many 200 mg/kg/day males, but neither persisted throughout the study. Some reproductive tissue weights were reduced in 12 and 200 mg/kg/day rats, but differences were comparatively slight at 12 mg/kg/day. Gross changes were limited to 200 mg/kg/day rats, except for the marginal change in mammary development at 12 mg/kg/day. Several reproductive structures were commonly not evident (seminal vesicle and coagulation glands) or reduced in size (prostate) at necropsy. Definitive histopathology was limited to 200 mg/kg/day rats. Testicular atrophy, epididymal azoospermia, and changes in several accessory reproductive tissues (chronic inflammation, hyperplasia, epithelial hyperplasia, and/or reduced secretion) were among the most common findings. NOEL = 6 mg/kg/day (areola/nipple development, organ weight decrements in seminal vesicles, coagulation glands, and prostate gland), with the findings at 12 mg/kg/day usually being transient and/or of small magnitude. Acceptable as an ancillary study. Study gives additional information about a previously identified possible adverse effect. Aldous, 7/9/98.

380-149 157019 (interim report of Record No. 160338, above - no worksheet)

380-144 155428 (interim report of Record No. 160338, above - a brief DPR worksheet was performed by Aldous on 7/24/97, which is superseded by the worksheet on the final report).

380-153 160339 Hellwig, J., C. Gembardt, and H. -P. Gelbke, "Reg. No. 83 258 - Pre-/postnatal toxicity study in Wistar rats after oral administration (gavage)," BASF A.-G., Ludwigshafen, 1/21/98, BASF Reg. Doc. # 98/10077. Female Wistar rats, 10/sex/group, were dosed by gavage with vinclozolin (99.4% purity) once daily from day 14 of gestation until day 3 *post partum*. Dose levels were 0, 3, 12, and 200 mg/kg/day. The primary objective was to evaluate feminization effects in male pups, such as altered anogenital distances (on days 2 and 22) and nipple development (on days 12 or 13 *postpartum*) resembling that of female pups. Pups were culled on day 2 by removing every other pup. Remaining male pups were maintained for about 180 days without breeding trials, then necropsied to evaluate reproductive structures and gross lesions. One F1 female/litter was also retained for the balance of the study, but these were not extensively studied. Treatment was well tolerated by dams. NOEL = 3 mg/kg/day (reversible areola/nipple development in 12 mg/kg/day male pups). These mammary gland changes were much more pronounced in 200 mg/kg/day males, where they persisted for at least 180 days. The high dose group suffered an increase of stillborn pups, plus an excess of deaths through the first 2 days *post partum*. Survival did not vary between groups after that time. High dose pup weights were significantly reduced throughout lactation and for the balance of the study. Anogenital distance was reduced in high dose males on days 2 and 22. Several changes in reproductive structures were evident in-life, all limited to 200 mg/kg/day. Of these, hypospadias was most universal and persistent. Paraphimosis, hypoplasia of penis, and hypoplasia of testis were observed in many 200 mg/kg/day males, with variable reversibility over time. Some reproductive tissue weights were reduced in 200 mg/kg/day rats, especially prostate and seminal vesicles. Gross changes were limited to 200 mg/kg/day rats. Several reproductive structures often could not be found at necropsy (seminal vesicle and coagulation glands). Histopathologic effects were limited to 200 mg/kg/day rats. Changes in several accessory reproductive tissues (chronic inflammation, hyperplasia, epithelial hyperplasia, and/or reduced secretion) were the most common findings. Acceptable as an ancillary study. Study gives additional information about a previously identified possible adverse effect. Aldous, 7/20/98.

380-150 157020 (interim report of Record No. 160339, above - no worksheet)

380-145 155429 (interim report of Record No. 160339, above - a brief DPR worksheet was performed by Aldous on 7/24/97, which is superseded by the worksheet on the final report).

REPRODUCTION - MECHANISM STUDIES

380-084 092438 van Ravenzwaay, B., "Report on the effect of Reg. No. 83 258 (ZST No. 88/375) on 5-alpha-reductase activity in vitro". BASF AG, Sept. 4, 1990. Development of some male organs such as the prostate are regulated largely by the testosterone product, 5-alpha-dihydrotestosterone, which is produced via reduction of testosterone by 5-alpha-reductase. This study attempted to determine whether activity of 5-alpha-reductase, obtained from supernatant of liver homogenates, would be inhibited by vinclozolin. No such inhibition could be demonstrated. Aldous, 3 July 91 (no SB-950 worksheet).

380-084 092439 Knuppen, R., "Final report: Examination of the hormone status associated with the six-month feeding study in Wistar rats". Project No. 31S0375/88050. Institute of Biochemical Endocrinology, Medical Univ. of Lübeck, July 1, 1989. Apparently plasma samples were analyzed from 10 controls/sex and 20 treated rats/sex for various hormones. The sketchy report does not indicate dose of treated rats. The most remarkable change was LH concentration increase with treatment: about 10-fold increase in males and about 2-fold increase in females. Additional highly statistically significant differences were elevated testosterone in males (females were not assayed), and elevated DHEA (dehydroepiandrosterone) in males only. Slightly statistically significant changes were elevated ACTH (both sexes), and corticosterone (slightly elevated in males only). Investigators presumed that the pituitary/gonadal axis was affected, however further clarification was expected following histology of the ongoing reproduction study. Aldous, 3 July 91 (no SB-950 worksheet).

380-084 092440 Knuppen, R. "Final Report: Study of the binding of 3-(3,5-dichlorophenyl)-5-methyl-vinyl-2,4-dione to the androgen receptor in MCF-7 cells". Project No. 21B 0324/889027. Institute of Biochemical Endocrinology, Medical Univ. of Lübeck, (original report in German signed by Knuppen on 4/27/90). This 11-pg translation did not include the protocol, which presumably described MCF-7 cells. It is apparent from the data provided that MCF-7 cells contain androgen receptors. There was considerable breakdown of vinclozolin in cytosol from MCF-7 cells, as well as in the cell culture medium (but major breakdown products were different in the two systems). Affinity to androgen receptor by vinclozolin in MCF-7 cytosol was about 1/1000 to 1/2000 compared to the synthetic androgen, mibolerone. Vinclozolin apparently did not compete with binding of estradiol to the estrogen receptor in MCF-7 cytosol. Apparently vinclozolin competed with mibolerone for binding sites in the nuclei of intact MCF-7 cells, with affinity about 1/4000 of mibolerone. The short translation did not include details of study design, nor tables nor figures of results, but provided some useful data. Aldous, 7/5/91, (No worksheet).

TERATOGENICITY, RAT (ORAL)

380-056 071710 "First preliminary results of several prenatal toxicity studies with REG. NO. 83 258 (Vinclozolin) in rats after oral administration". BASF AG, Ludwigshafen, FRG. Date of preliminary report: 10/18/88. These preliminary results were reviewed by C. Aldous, 12/20/88. See reviews of the completed reports from Document 380-063, below.

380-063 074654 Hellwig, J., "Report on the Study of the Prenatal Toxicity of Reg. No. 83 258 in Rats After Oral Administration (Gavage) - First Study" (BASF Department Of Toxicology, Study 89/0090, 3/23/89) Reg. No. 83 258, batch N173, 99.6%, was given to Wistar rats by gavage at treatment levels of 0 (0.5% carboxymethyl cellulose), 15, 50 or 150 mg/kg/day, 25/group. NOEL (maternal) > 150 mg/kg/day (HDT). NOEL (developmental) = 15 mg/kg/day. At 50 and 150 mg/kg/day male fetuses had a statistically significant reduction in anogenital distance. Further studies are needed to determine if this is a real teratogenic effect. This apparent feminization is **a possible adverse effect. Unacceptable as an independent report** (selected dose levels were not justified). D. Shimer/ C. Aldous, 8/23/90.

380-063 074655 Hellwig, J., ""Report on the Study of the Prenatal Toxicity of Reg. No. 83 258 in Rats After Oral Administration (Gavage) - Second Study" (BASF Department of Toxicology, Document No. 89/0091, 3/23/89). Vinclozolin/Reg. No. 83 258, Batch N 173, 99.6%, was administered to Wistar rats by gavage at 0 (0.5% carboxymethyl cellulose), 50, 100 or 200 mg/kg/day, 25/group, on days 6 - 19 of gestation. NOEL (maternal) = 200 mg/kg/day (HDT), no maternal effects were seen. NOEL (developmental) = 100 mg/kg/day, there was a slight increase in the numbers of fetuses with retarded ossification of thoracic vertebral bodies: this finding is consistent with observations at comparable or higher dose levels in the studies reported in this volume. NOTE: fetal anogenital distances were not measured. **No adverse effect. Unacceptable.** Dose levels did not demonstrate maternal toxicity. D. Shimer/ C. Aldous, 8/23/90.

380-063 074656 Hellwig, J., "Report on the Study of the Prenatal Toxicity of Reg. No. 83 258 in Rats After Oral Administration (gavage) - Third Study" (BASF Department of Toxicology, BASF document No. 89/0092, 3/23/89). Reg. No. 83 258 (Technical. Vinclozolin), Batch N 173, 99.6%, was administered to presumed pregnant Wistar rats at 0 (0.5% carboxymethyl cellulose), 200 or 400 mg/kg/day, 25/group, days 6-19 of gestation. NOEL (maternal) = 400 mg/kg/day, no effects were seen. No developmental NOEL was obtained in this study: there was a statistically significant decrease in the anogenital distance of male fetuses, increased incidences of dilated renal pelvis and of hydroureter, and an increase in the number of fetuses with retarded ossification of thoracic vertebral bodies. These findings constitute **possible adverse effects. Unacceptable as an independent report**, (dose levels did not demonstrate maternal toxicity). D. Shimer/C. Aldous, 8/23/90.

380-063 074657 Hellwig, J., "Report on the Study of the Prenatal Toxicity of Reg. No. 83 258 in Rats after Oral Administration (Gavage)" (BASF Department of Toxicology, Document No. 89/0093, 3/23/89). Reg. No. 83 258, Batch N 173 (Vinclozolin), 99.6%, was given to Wistar rats by gavage at treatment levels of 0 (0.5% carboxymethyl cellulose), 600 or 1000 mg/kg/day, 10/group. **Maternal** NOEL < 600 mg/kg (not established in this study). At 600 mg/kg/day, food consumption was temporarily reduced, water intake was appreciably increased, unsteady gait was observed in one dam (this sign was common at 1000 mg/kg/day), and liver and adrenal weights were statistically significantly ($p < 0.01$) increased. Total litter resorptions were noted in 2 females at 600 mg/kg/day: this might have represented a treatment effect, however there was no evidence of embryo/fetal lethality at 1000 mg/kg/day. **Developmental** NOEL < 600 mg/kg (not established in this study). The most characteristic effect was markedly reduced ano-genital distance in males at both treatment levels. Hydroureter incidence was elevated in both treated groups (both sexes). The above findings constitute **possible adverse effects. Unacceptable as an independent study**: (too few animals per group were used, NOEL was not established). D. Shimer/C. Aldous, 8/23/90.

****380-063 [Record Nos. 074654 to 074657:** (Four rat teratology studies being considered together)]. All studies by BASF Department of Toxicology, all dated 3/23/89. The maternal NOEL was 400 mg/kg/day (based on Record Nos. 074656 and 074657). The developmental NOEL was 15 mg/kg/day (based on Record No. 074654). Only one study (Record No. 074657) demonstrated maternal toxicity. The latter study employed fewer than the numbers of dams recommended by guidelines, however there were no indications of unanticipated effects at dosages nearly two orders of magnitude above the developmental effects NOEL, hence a meaningful range of dosages was evaluated. Taken collectively, the data support filling the rat teratology data gap. The primary indication of a **"possible adverse effect"** in these studies was decreased anogenital distance in males. Aldous, 8/23/90.

380-060:072957, 072958, 072959, and 072960 (Appendices 6-9) Protocols for rat teratology studies which were later reported in Document 380-063 (see above).

380-056 071712 Takehara, K., M. Itabashi, T. Inoue, and M. Tajima, "Teratogenicity study of Vinclozolin (BAS-352F) to rats in dietary administration". Nippon Institute for Biological Science, Dec., 1979. Vinclozolin, technical., 92.8%, was administered in diet at 0, 300, 1500, and 7500 ppm (average calculated dosages 0, 23, 111, and 394 mg/kg/day) to CD/CRJ rats (18 to 22 pregnant rats/group) on days 0-21 of gestation. **Possible adverse effect:** decreased anogenital distance (male pseudohermaphroditism) in males at 1500 and 7500 ppm. Maternal NOEL = 300 ppm (transient decreases in food and water intake, slight decrease in body weight, enlarged adrenals). Developmental NOEL = 300 ppm (decreased anogenital distances in males, increased incidence of left lumbar ribs). 7500 ppm group had marked maternal weight gain decrements, and clinical signs such as apparent weakness, depression, and discolored hair. Ovaries were enlarged compared to other groups. Fetuses of 7500 ppm group were significantly smaller in size, had ossification delays, and had increased incidence of hydronephrosis and dilated ureters. This group had 3 total litter losses. Study **not acceptable**, but useful data. Upgradeability is a moot issue, since studies more consistent with guidelines have been accepted. C. Aldous, 12/7/88, (update by Aldous, 8/23/90: no new worksheet).

TERATOGENICITY, RAT (DERMAL)

NOTE: The rat teratology study data gap is filled, so that "acceptability" is not an issue for the following studies. The following dermal exposure studies provide some useful data by evaluating the extent of developmental effects following a route of possible human exposure. Even with a rather aggressive study design (treatment through day 20 in Record No. 139083), the NOEL was a comparatively high 30 mg/kg/day. Aldous 11/27/95.

380-068 086144 Hellwig, J., "Study of the prenatal toxicity of Reg. No. 83 258 in rats after dermal application" (Project No. 34RO375/88074). BASF AG, Dept. of Toxicology, Ludwigshafen, FRG. Feb. 1, 1990. Wistar rats, 25/group, were given doses of 0, 60, 180, or 360 mg/kg/day dermally for 6 hr/day, days 6 to 19 p.c., as suspensions in 0.5% carboxymethyl cellulose. A **possible adverse effect was indicated** for developmental effects: **Developmental NOEL** = 60 mg/kg/day (modest, but statistically significant ($p < 0.01$) decreases in anogenital distances in male fetuses). **Maternal NOEL** = 60 mg/kg/day (modest, but statistically significant ($p < 0.01$) increases in adrenal gland weights). The study is **unacceptable**, and not upgradeable (no overt maternal toxicity). The modest degree of response in anogenital distances in males is consistent with preliminary results of a dermal

exposure study (380-073:088748, no written review of this study has been done nor is one expected to be done by Medical Toxicology Branch), which indicated that the percent of dermal absorption decreases with increasing dose concentrations, so that less than one percent of applied dose would be absorbed at the highest dose level in the dermal teratology study. Aldous, 8/22/90. Note: EPA review of 3/28/90 identified the same NOEL's, based on the same pivotal findings indicated above. EPA Core classification is "Supplementary" (stability data are needed).

380-134 139083 Hellwig, J., "Study of the prenatal toxicity of Reg. No. 83 258 in Wistar rats after dermal application", BASF Aktiengesellschaft, Ludwigshafen, 4/27/95. Lab. Project ID 34R0375/88124. Mated Wistar rats (Chbb:THOM (SPF)), 25/group, were dosed dermally (occlusive dressing) for 6 hr/day on p.c. days 6-20 with 0, 10, 20, 30, or 200 mg/kg vinclozolin (99.3%). Rats were sacrificed on p.c. day 21. Reproduction parameters were evaluated, however examinations of fetuses were limited primarily to anogenital distance measurements. Serum samples were taken from five dams/group and from all fetuses of 5 litters/group (pooled by litter), for assays of vinclozolin and of its two major metabolites. The NOEL for developmental changes assessed in this study was 30 mg/kg/day, based on an increase in numbers of 200 mg/kg/day males with anogenital distances in the range of 2.5 to 2.9 mm (the lower end of the normal range for normal males), although the mean anogenital distances did not differ significantly between groups at $p < 0.05$. Maternal toxicity NOEL = 200 mg/kg/day. Vinclozolin was not detected in sera of dams or fetuses, but one of the metabolites was detected at 200 mg/kg/day in dams and fetuses (about 60% higher concentrations in fetal serum than in serum of dams). This study provides a dermal exposure NOEL for the primary fetal effect during the most sensitive prenatal period (late gestation), hence is useful ancillary information. No adverse effect is indicated. Aldous, 11/27/95.

TERATOGENICITY, MOUSE

380-027 035321 "Study on the prenatal toxicity of 3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidin-2,4-dione on mice". BASF, 2/18/75. Vinclozolin (no purity stated); given in the diet to 30/group at 0, 600, 6000, and 60,000 ppm on days 0-18 of gestation. All dams in high dose died. **Possible adverse effect indicated:** No implants in 6000 ppm group. No maternal or fetal effects at 600 ppm. Unacceptable (only one dose level with fetuses to evaluate for developmental toxicity), not upgradeable. NOEL = 600 ppm (developmental), NOEL = 6000 ppm (maternal). Adverse effects were not identified in initial review, but re-evaluation (6/13/86) identified possible adverse effect (embryotoxicity at 6000 ppm). J. Parker, 11/15/85, 6/13/86.

005 981452 Partial duplicate of 035321. Reviewer [J. R. (Gee), 4-18-85] noted reproductive toxicity as possible adverse effect.

TERATOGENICITY, RABBIT

****380-070 087082, 087083** "Report on the study of the prenatal toxicity of Reg. No. 83 258 (Vinclozolin) in rabbits after oral administration (gavage)". Project No.: 38R0375/88062. BASF AG, Department of Toxicology, Ludwigshafen, FRG. Reports dated 2/14/90 (main study), and 2/22/90 (supplementary study). Himalayan rabbits were dosed by gavage with 10 ml/kg 0.5% CMC suspensions of vinclozolin from days 7 to 28 p.c. Numbers of dams/dose in the main study

were 15/group at 0, 50, 200, and 800 mg/kg/day. The supplementary study involved 20/group at 0 and 400 mg/kg/day. Data are evaluated here as a single report. **Maternal NOEL = 50 mg/kg/day** (at 200 mg/kg/day, dams had slightly decreased food consumption; statistically significantly ($p < 0.01$) increased liver weights; also one dam aborted following clinical signs of "reduced defecation" and "reddish-brown discoloration of urine": these were common observations in higher dosage groups). **Developmental NOEL = 200 mg/kg/day** (2/10 dams had total litter resorptions at 400 mg/kg/day). At 400 and 800 mg/kg/day, there were high incidences of maternal deaths and abortions; clinical signs of reduced or absent defecation, or of red-brown discolored urine were common; food consumption was more markedly decreased; and increased reticulocyte counts suggested mild anemia. **No adverse effect, acceptable.** Aldous, 8/23/90.

380-070 087083 [Text of supplemental study to 087082, above; also data tables for both the main study and the supplementary study]. Gelbke, H. P., "Report on the Supplementary Study of the prenatal toxicity of Reg. No. 83 258 (Vinclozolin) in rabbits after oral administration (gavage). Project No.: 38R0375/88062". This supplemental study involved one control group and one treated group (400 mg/kg/day). The two records were reviewed as a single entity. Aldous, 8/23/90.

380-060 072961 Protocol for Project # 38R0375/88062 (070:087082, above).

380-027 035322 "Effect of Vinclozolin on pregnancy of the New Zealand White rabbit". HRC, (Audit 6/3/81). 0, 20, 80, and 300 mg/kg/day Vinclozolin (purity stated by supplier to be 98.1%) by gavage as suspension in 1% methylcellulose. Fifteen/group assigned to study: 10 to 13 per group with viable young at termination. **Unacceptable: no adverse effects indicated** (No parental or developmental toxicity up to 300 mg/kg/day (HDT) in the primary study). Study was rejected by CDFA on 11/18/85 due principally to inadequacy of dosage range in the primary study. Study was re-examined on 10/11/88, considering HRC rebuttal comments (041:060671). There was no change in study status. J. Parker (11/18/85) and C. Aldous (10/11/88).

041 060671 Statement dated 6/15/87 on maternal toxicity by contracting lab (HRC) Study Director, D.D. Cozens and Principal Toxicologist of Reproductive Studies, A.K. Palmer with respect to 027:035322, above. The lab defends 300 mg/kg/day as an appropriate high dose level, and indicates that a three-fold increase above this dose would have been clearly too high. (Statement considered in 10/11/88 review).

GENE MUTATION

380-027 035313 Englehardt, G. and H. P. Gelbke, "Report on the study of Vinclozolin (Reg. No. 83 258) (ZNT Test Substance No.: 82/370) in the Ames test (Standard plate test and preincubation test with Salmonella typhimurium)". BASF, 8/1/83. Vinclozolin (98%) tested at 0, 100, 500, 2500, 5000, 7500 or 10,000 µg/plate with and without Aroclor induced rat liver activation; precipitation at 5000 and above; Salmonella strains TA 98, 100, 1535, 1537 and 1538; also used liquid preincubation method for TA 100 only; no mutagenicity reported; unacceptable (repeat experiment with TA 100 only), not upgradeable. J. Remsen (Gee), 10/15/85.

380-027 035318 Shirasu, Y., M. Moriya, and K. Kato, "Mutagenicity Testing on BAS-35204F in Microbial Systems" (Inst. Environ. Toxicology., 12/27/77) Vinclozolin (92.8%) tested on

Salmonella G46 in a host-mediated assay with 6 male ICR mice/concentration given 2 doses by oral gavage and Salmonella injected i.p.; 0, 200, 400, 1000 and 2000 mg/Kg; mice were sacrificed 3 hours after injection of Salmonella; no evidence of mutagenicity: unacceptable (dose levels not high enough, no evidence of exposure of test organisms), cannot be upgraded. Remsen 10/15/85, Davis 10/17/88

380-041 060672 Statement by the conducting laboratory (Inst. Environ. Toxicology.) giving dose selection rationale and the Summary from "The metabolic fate of the fungicide Vinclozolin, BAS 352F, after repeated oral administration to rats" (Huntingdon Research Centre, England, 11/17/76). (Refers to 380-027 035318, above). Davis 10/17/88.

380-027 035319 Shirasu, Y., M. Moriya, and K. Kato, "Mutagenicity testing on BAS-35204F (Vinclozolin) in microbial systems - Reverse mutation with Escherichia coli and Salmonella typhimurium". Inst. Environ. Toxicology., 2/20/78. Vinclozolin (92.8%) tested +/- rat liver S9 at 0, 1, 5, 10, 50, 100, 500, 1000 or 3000 µg/plate on Salmonella strains TA 98, 100, 1535, 1537 and 1538; no increased reversion reported; unacceptable (no repeat trial), not upgradeable. J. Remsen (Gee), 10/15/85.

380-154 160341 Engelhardt, G. and H. D. Hoffmann, "Salmonella typhimurium reverse mutation assay with norharman: Two screening studies for the comparison of 3,5-dichloroaniline and 4-chloroaniline", BASF, Ludwigshafen, 8/29/97. BASF Reg. Doc. No. 97/11005. These two chloroanilines, both of purity $\geq 99\%$, were tested in the Ames-style Salmonella TA 98 plate assay with and without S-9. Under these conditions, both articles were negative for mutagenicity. Norharman is known to facilitate mutagenic response of some aromatic amines. When norharman and S-9 were present in the system, 4-chloroaniline but not 3,5-dichloroaniline elicited a dose-related increase in revertants. Only 3,5-dichloroaniline is an analog of vinclozolin. Data indicate caution in generalizing about the potential mutagenicity of various chloroanilines, and that the 3,5-dichloroaniline product which may arise from vinclozolin metabolism is not indicated as a mutagen in this assay. Valid ancillary study. Aldous, 7/13/98.

Mammalian Systems

****380-027 035316** Witterland, W. F., "Mutagenicity evaluation of Vinclozolin (83/233) in the mouse lymphoma forward mutation assay". Litton Bionetics, 6/84. Vinclozolin (no purity stated) tested +/- S9 (rat liver) at 0, 10, 25, 50, 75, 100, 250, 500 or 1000 µg/ml (precipitate above 150) on mouse lymphoma L5178Y exposed for 4 hrs; 2 or 3 repeat trials; POSSIBLE ADVERSE EFFECT with increased mutation frequency with S9 only, an increase of 2 to 3 fold; ACCEPTABLE. J. Remsen (Gee), 10/15/85.

380-041 060673 (Litton Bionetics, Netherlands, 7/8/87) Statement by the conducting laboratory emphasizing the uncertainty of the positive results. B. K. Davis, 10/17/88.

380-034 042905 Jäckh, R. and H.-P. Gelbke, "Report on a point mutation test carried out on CHO cells (HGPRT locus) with the test substance Vinclozolin (Substance No. 84/382)" (BASF Aktiengesellschaft, Report No: 85/352, 10/29/85) Chinese hamster ovary cells (CHO-K1) exposed in quadruplicate to vinclozolin (99.5%) at 0, 0.316, 1.0, 3.16, and 10.0 mg/ml; 4 hours; \pm S9; NO ADVERSE EFFECT; UNACCEPTABLE: No confirmatory assay although results were ambiguous. J. Carlisle 7/30/86, B. K. Davis 10/13/88.

SUMMARY OF GENE MUTATION STUDIES: Although record #'s 035313 and 035319 are individually unacceptable, primarily because each lacks a repeat, confirming trial; taken together they are adequate to fill the gene mutation test type in bacteria. The data gap, however, is actually filled by the study # 035316 in mouse lymphoma cells. No adverse effect was identified in *Salmonella* but a weak mutagenic effect was found in the mouse cells. While the Chinese hamster ovary cell assay (# 042905) was reported negative, there was ambiguity in the results (see the peer review worksheet by B. K. Davis 10/13/88). In view of the inadequacy of a number of the in vivo (rat and mouse combined) tests, the positive finding in mammalian cell mutagenicity cannot be dismissed. Summary by J. Remsen (Gee), approx. 10/15/85; update by B. K. Davis, approx. 10/17/88.

CHROMOSOME EFFECTS

****380-027 035320** Englehardt, G. and H. P. Gelbke, "Cytogenetic investigations in Chinese hamsters after a single oral administration of Reg. No. 83 258 (Vinclozolin): Sister chromatid exchange". BASF, 1/15/82. Vinclozolin (98.1%) tested in sister chromatid exchange (in vivo) with Chinese hamsters; 0, 3830 or 5620 mg/kg administered orally in one dose; negative and positive controls; 4/sex/group; BudR tablet implanted subcutaneously 2 hr before dosing; sacrificed at 24 hours; no increase in SCE reported; ACCEPTABLE. J. Remsen (Gee), 10/15/85.

380-027 035312 Hofmann, H. T. and J. Peh, "Study of the mutagenic effect of 3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazoladin-2,4-dione on the male mouse following repeated oral administration". BASF, 2/18/75. Vinclozolin (no purity stated); tested at 0 or 2000 mg/kg/day by oral gavage for 5 days in mouse dominant-lethal assay; 20 or 21 males per group; mated 1:3 for 8 weekly periods; no evidence of a dominant lethal effect. NOEL greater than 2000 mg/kg. Unacceptable (no concurrent positive control or historical data, single dose level only with no evidence of toxicity, no individual data, test substance not characterized), not upgradeable. J. Remsen (Gee), 12/6/85.

380-005 981460 Partial duplicate of 035312.

380-005 981461 Record Number not found, but title is same as 035312 above.

****380-140 147392** Engelhardt, G., "Cytogenetic study *in vivo* of vinclozolin (Reg. No. 83 258) in NMRI mice micronucleus test single intraperitoneal administration", BASF AG, Ludwigshafen, 12/18/95. Sponsor Report No. 95/11176. Five mice/sex were dosed once ip with 0, 375, 750, or 1500 mg/kg vinclozolin 24 hr prior to sacrifice, or with 0 or 1500 mg/kg vinclozolin 48 hr before sacrifice. Femur bone marrow preparations were evaluated for micronuclei in PCE's. No treatment effect was found. Positive controls (cyclophosphamide and vincristine) proved functional. Study is acceptable with no adverse effect. Aldous, 7/16/97.

****380-140 147393** Engelhardt, G., "Cytogenetic study *in vivo* of vinclozolin (Reg. No. 83 258) in CD-1 mice micronucleus test single intraperitoneal administration", BASF AG, Ludwigshafen, 12/18/95. Sponsor Report No. 95/11174. Five mice/sex were dosed once ip with 0, 375, 750, or 1500 mg/kg vinclozolin 24 hr prior to sacrifice, or with 0 or 1500 mg/kg vinclozolin 48 hr before sacrifice. Femur bone marrow preparations were evaluated for micronuclei in PCE's. No treatment effect was found. Positive controls (cyclophosphamide and vincristine) proved functional. Study is acceptable with no adverse effect. Aldous, 7/16/97.

[No DPR document or record number: not a registrant submission]. Hrelia, P. *et al.* (1994), "A battery of biomarkers for detecting carcinogenic risk from fungicides", *Clinical Chemistry* **40**: 1460-1462. Report contains one small table, which shows an increase in micronuclei/1000 PCE's compared to the concurrent control (1.30 for control vs. 5.25 for 1250 mg/kg ip dose of vinclozolin). The same table contains data for fenarimol, with its control yielding 5.02 micronuclei/1000 PCE's. This control value, nearly identical to the above vinclozolin result, casts doubt on the importance of the statistically significant vinclozolin result. Vinclozolin increased activity of liver *p*-nitrophenol hydroxylase 5-fold following repeated dosing with 750 mg/kg/day. This was noted to potentially enhance toxicity of certain xenobiotics. Study is unacceptable (too little information to be properly evaluated). Upgrade is possible, but not likely nor needed (there are already 3 accepted studies for chromosomal effects). Aldous, 6/27/97.

DNA EFFECTS

****380-027 035314** Hoorn, A. J. W., "Mutagenicity of Vinclozolin in the Rec - assay with Bacillus subtilis". Litton Bionetics, 12/83. Vinclozolin (no purity stated); tested at 0, 1, 10, 100, 500, 1000, 2500 or 10,000 µg/well on Bacillus subtilis H17 (rec⁺) and M45 (rec⁻); 3 replicates per concentration; no evidence of adverse effect by zone of inhibition; ACCEPTABLE. J. Remsen (Gee), 10/15/85.

380-027 035317 Shirasu, Y., M. Moriya, and K. Kato, "Mutagenicity testing on BAS-35204F (Vinclozolin) in microbial systems - Rec-assay on Bacillus subtilis". Inst. Environ. Toxicology., 2/20/78. Vinclozolin (92.8%) tested at 0, 20, 100, 200, 500, 1000 or 2000 µg/disc on Bacillus subtilis H17 and M45 without metabolic activation; no zone of inhibition reported for either strain; not acceptable (no trials with metabolic activation, no mention of number of plates per concentration or of precipitation problems), not upgradeable. J. Remsen (Gee), 10/15/85.

****380-027 035315** Cifone, M. A., "Evaluation of Vinclozolin in the primary rat hepatocyte unscheduled DNA synthesis assay". Litton Bionetics, 1/84. Vinclozolin (>99.5%) tested at 0, 5, 10, 25, 50, 100, 250, 500 or 1000 µg/ml for UDS in primary hepatocytes from a Fischer 344 rat; 18 hr exposure; 3 replicates per assay, 50 cells per coverslip, 2 cultures for cytotoxicity; no evidence of positive response; ACCEPTABLE. J. Remsen (Gee), 10/15/85.

MUTAGENICITY-RELATED MISCELLANEOUS REPORTS

380-154 160340 Lam, W., "Structure reactivity consideration of 4-chloroaniline and 3,5-dichloroaniline", BASF Corporation, 1/98. This is a discussion emphasizing the differences between 4-chloroaniline and 3,5-dichloroaniline, which would affect respective mutagenic potentials. 3,5-dichloroaniline has a lower pKa than 4-chloroaniline, which would be expected to make the former less reactive toward bioactivation to aryl nitrenium ions (postulated to be important ultimate oncogenic and mutagenic species). 3,5-dichloroaniline is 3 orders of magnitude less reactive toward H₂O₂ in the presence of horseradish peroxidase than 4-chloroaniline. There is a comparable difference in their respective "hemoglobin binding indices". These observations suggest that the differences between these two anilines are substantial, and therefore derivatives of these two structures should not be expected to be similar in biological responses. No reviewable data. No DPR worksheet. Aldous, 7/10/98.

380-154 160342 Sabbioni, G. and O. Sepai, "Comparison of hemoglobin binding, mutagenicity, and carcinogenicity of arylamines and nitroarenes", **Chimia** **49**:374-380 (1995). Investigators noted that N-hydroxyarylamines often lead to mutagenic products, which form adducts with DNA. Further, hemoglobin adducts with various arylamines could serve as a dosimeter of exposure. In the case of *ortho* or *meta* halogen-substituted arylamines, hemoglobin binding was found to be directly proportional to the pK_a . This relationship had been considered by Lam (above) in concluding that 4-chloroaniline should have a greater capacity to form mutagenic products than would 3,5-dichloroaniline. No worksheet. Aldous, 7/13/98.

METABOLISM

380-069 088542 "The biokinetics and metabolism of ^{14}C -Vinclozolin in the rat: Interim Report". Huntingdon Research Centre Ltd., 12/7/89. Wistar rats were dosed with [phenyl ring-labeled] ^{14}C -Vinclozolin (^{14}C -V), either (1) by single gavage dose of 10, 100, or 200 mg/kg/day ^{14}C -V to monitor plasma levels, or (2) by single gavage dose of 10 or 100 mg/kg/day ^{14}C -V (or single gavage dose of 10 mg/kg/day ^{14}C -V to rats pre-dosed with 10 mg/kg/day unlabeled Vinclozolin for 14 days) to evaluate urine and feces levels, or (3) by single iv dose of 1 mg/kg/day ^{14}C -V to evaluate urine and feces levels. Gavage administration led to nearly complete recovery in urine and feces (no detectable ^{14}C was trapped in expired air). Generally, slightly more label was found in urine than in feces. Amounts administered, or presence or absence of pretreatment, did not greatly affect excretion patterns. Intravenous treatment led to about 70-73% of label in urine vs. 23% in feces. Residues in tissues after 5 days were low: typically tissue levels (on equivalents/g tissue basis) were less than 1% of equivalents/g b.w. administered [exceptions were liver and kidney (M and F), and fat (F): in these tissues, residues up to 2-3 % of equivalents/g b.w. administered were found]. Metabolites were not identified in this preliminary report. Patterns were similar in the sexes, except that females had larger amounts of one late-eluting component (Fig. 4). **Not acceptable** (interim report): useful information. Aldous, 8/23/90.

380-084 092441 Hawkins, D.R., et al. "The biotransformation of ^{14}C -vinclozolin in the rat". Huntingdon Research Centre, Ltd., Cambridgeshire, 11/29/90. HRC Report No. HRC/BSF 479/901497. (See above for an earlier report). The major metabolite in urine, feces, and bile was N-(3,5-dichlorophenyl)-2-methyl-2,3,4-trihydroxybutyramide (in the case of urine and bile, this compound was conjugated as the glucuronide). The percentage of recovered label found in bile of cannulated rats was variable, but ranged as high as 71% of recovered radioactivity. Comparatively high tissue levels were found in liver and kidney. In either tissue, N-(3,5-dichlorophenyl)-2-methyl-2,3,4-trihydroxybutyramide was the major metabolite (not conjugated). Aldous, July 5, 1991, (No worksheet: not a study type required under SB-950: useful data).

380-084 092442 Hawkins, D.R. et al. "The biokinetics of ^{14}C -Vinclozolin in the rat". Huntingdon Research Centre, Ltd., Cambridgeshire, 11/28/90. HRC Report No. HRC/BSF 477/90916. This is an extensive, QA Unit-approved report with GLP signoff (153 pp.): some of the data here were reported in 380-069 088542 (above). Data not already summarized in 1-liners above include: plasma half-lives following single oral doses of 10 to 200 mg/kg/day vinclozolin were estimated at 23 and 36 hr for M and F, respectively. Similarly, half life after a 12-hr peak plateau following dietary administration was about 40 hr. Aldous, July 5, 1991, (No worksheet: not a study type required under SB-950).

380-084 092443 Hawkins, D.R. et al., "The dermal absorption of ^{14}C -Vinclozolin in the rat". Huntingdon Research Centre, Ltd., Cambridgeshire, Jan. 3, 1991. Study designation HRC/BSF 478/901583. Male Wistar rats were dosed with 0.002, 0.02, 0.2, or 2 mg vinclozolin/cm² skin (nominal doses of 0.13, 1.3, 13, or 130 mg vinclozolin/kg b.w.) in CMC vehicle on shaved, unabraded skin of the back for up to 10 hr or until time of sacrifice (if earlier than 10 hr), at which time excess material was washed off with water. Rats were sacrificed at 0.5, 1, 2, 4, 10, or 72 hr after application. Total absorbed was defined as the sum of % of administered dose recovered in urine, cagewash, feces, and tissues plus remaining carcass. Percent absorption went down with increased dose, so that at 72 hr, total percent absorbed dose in 0.002, 0.02, 0.2, or 2 mg/cm² groups was 27, 20, 3, and 1%, respectively. In general, urine accounted for most of the label recovered. Feces generally contained about half as much label as urine for a given group. Most of the label was removed in the washing step in all groups, but the highest percentage recovery in washing was obtained from the higher dose animals. Report provides QA and GLP conformance statements. Aldous, July 8, 1991, (No worksheet: not a study type required under SB-950).